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Neuroprotective effects of the allosteric agonist of metabotropic glutamate receptor 7 AMN082 on oxygen-glucose deprivation- and kainate-induced neuronal cell death

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ABSTRACT

Although numerous studies demonstrated a neuroprotective potency of unspecific group III mGluR agonists in in vitro and in vivo models of excitotoxicity, little is known about the protective role of group III mGlu receptor activation against neuronal cell injury evoked by ischemic conditions. The aim of the present study was to assess neuroprotective potential of the allosteric agonist of mGlu7 receptor, *N,N'-Bis*(diphenylmethyl)-1,2-ethanediamine dihydrochloride (AMN082) against oxygen-glucose deprivation (OGD)- and kainate (KA)-evoked neuronal cell damage in primary neuronal cultures, with special focus on its efficacy after delayed application. We demonstrated that in cortical neuronal cultures exposed to a 180 min OGD, AMN082 (0.01–1 μM) in a concentration- and time-dependent way attenuated the OGD-induced changes in the LDH release and MTT reduction assays. AMN082 (0.5 and 1 μM) produced also neuroprotective effects against KA-evoked neurotoxicity both in cortical and hippocampal cultures. Of particular importance was the finding that AMN082 attenuated excitotoxic neuronal injury after delayed application (30 min after OGD, or 30 min–1 h after KA). In both models of neurotoxicity, namely OGD- and KA-induced injury, the neuroprotective effects of AMN082 (1 μM) were reversed by the selective mGlu7 antagonist, 6-(4-Methoxyphenyl)-5-methyl-3-(4-pyridinyl)-isoxazolo[4,5-*c*]pyridin-4(5*H*)-one hydrochloride (MMPIP, 1 μM), suggesting the mGlu7-dependent mechanism of neuroprotective effects of AMN082. Next, we showed that AMN082 (0.5 and 1 μM) attenuated the OGD-induced increase in the number of necrotic nuclei as well inhibited the OGD-evoked calpain activation, suggesting the participation of these processes in the mechanism of AMN082-mediated protection. Additionally, we showed that protection evoked by AMN082 (1 μM) in KA model was connected with the inhibition of toxin-induced caspase-3 activity, and this effect was abolished by the mGlu7 receptor antagonist. The obtained results indicated that the activation of mGlu7 receptors may be a promising target for neuroprotection against ischemic and excitotoxic insults.

1. Introduction

Glutamate is the main excitatory neurotransmitter in the mammalian brain (Headley and Grillner, 1990). It acts on different receptor types: ionotropic glutamate receptors (iGluRs) (NMDA, AMPA, and kainate receptors) and metabotropic glutamate receptors (mGluRs). It is well established that glutamatergic overactivation may lead to neurodegeneration by the mechanism of excitotoxicity, a phenomenon that occurs e.g. under ischemic conditions (Choi, 1994; Lai et al., 2014; Olney, 1978; Olney and Ishimaru, 1999; Puyal et al., 2013).

A lot of studies have provided unequivocal evidence that glutamate-mediated excitotoxicity is a key player in cell death both after oxygen-glucose deprivation (OGD) in vitro and in experimental focal cerebral ischemia in vivo (Fujimoto et al., 2004; Lai et al., 2014; Prass and Dirnagl, 1998; Puyal et al., 2013). In vitro studies demonstrated acute and delayed neuronal damage after OGD (Fujimoto et al., 2004; Nuñez-Figueredo et al., 2014) with dirsruption of ionic homeostasis which lead to depolarization of neurons and release of excitatory aminoacids, most notably glutamate (Canas et al., 2006; Huang et al., 2009; Velly et al., 2003). Overstimulation of glutamate receptors (particularly NMDARs, and also AMPA/kainate receptors) induces a massive increase in intracellular Ca^{2+} concentrations, release of K^+ into the extracellular space, and cell swelling due to the passive movement of water with Na^+ influx. Consequently, the massively increased intracellular second messenger Ca^{2+} triggers numerous deleterious processes, including free radial formation and membrane degradation, mitochondrial dysfunction,

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inflammation, activation of various enzymes e.g. caspases, calpains, liposomal proteases, and endonucleases, DNA fragmentation and finally causes neuronal cell death by necrosis and/or apoptosis (Choi, 1994; Grammer et al., 2008; Lipton, 1999; Minnerup et al., 2012; Prass and Dirnagl, 1998). Within the ischemic cascade, many molecular targets can be pharmacologically modulated to produce neuroprotection and glutamate excitotoxicity is one of such targets. It has been shown that both the apoptotic and necrotic mechanisms of glutamate-induced neuronal death were blocked by antagonists of NMDA or AMPA/KA receptors (Belayev et al., 1995; Kawasaki-Yatsugi et al., 1997; Kochhar et al., 1991; O'Neill et al., 1998, 2000). Although numerous studies have demonstrated neuroprotective effects of the antagonists of iGluRs in animal models of ischemia, the results of clinical trials were unsuccessful (Caraci et al., 2012; Liu et al., 2012; Xu and Pan, 2013) due to adverse effects, such as ataxia, sedation, psychotic effects, and memory impairment (Danysz and Parsons, 1998; Ikonomidou and Turski, 2002; Muir and Lees, 1995).

A growing body of evidence suggests that an indirect modulation of the neurotoxic glutamatergic transmission is a promising strategy of neuroprotection. Such indirect modulation can be achieved by compounds acting on mGluRs (Bruno et al., 2001; Byrnes et al., 2009; Lea and Faden, 2003; Nicoletti et al., 1996). The mGluRs are G-protein coupled receptors that have been classified into three groups (I–III) on the basis of their sequence homology, signal transduction pathways and pharmacological profiles (Ferraguti and Shigemoto, 2006; Pin and Duvoisin, 1995). Group I mGluRs (containing mGlu1 and mGlu5) are positively coupled to phospholipase C through G_q protein and their activation leads to phosphoinositide hydrolysis and intracellular mobilization of Ca^{2+} ions. Receptors of group II (mGlu2 and mGlu3) and group III (mGlu4, mGlu6, mGlu7, and mGlu8) are negatively coupled to adenylyl cyclase through G_i/G_o proteins, and their activation leads to the inhibition of the cAMP formation (Conn and Pin, 1997; Spooren et al., 2003).

Group III mGlu receptors are localized predominantly on presynaptic terminals of glutamatergic and GABAergic neurons, where they are involved in the regulation of synaptic transmission (Conn and Pin, 1997). Besides presynaptic, a postsynaptic localization of these mGluRs has also been described (Bradley et al., 1996). It has been shown that the activation of presynaptic group III mGlu receptors located at the glutamatergic nerve terminals causes a decrease in glutamate release, thus inhibiting glutamatergic excitatory transmission (Cartmell and Schoepp, 2000; Schoepp, 2001). Hence, it has been suggested that the activation of these receptors may have neuroprotective effects. Indeed, a number of data have confirmed the neuroprotective properties of group III mGluR agonists against excitotoxicity evoked by NMDA, quinolinic acid, kainate (KA) or homocysteic acid in different animal models in vitro (Bruno et al., 1996, 2000; Domin et al., 2014; Gasparini et al., 1999; Iacovelli et al., 2002; Lafon-Cazal et al., 1999) and in vivo (Bruno et al., 2000; Domin et al., 2014; Folbergrová et al., 2008; Gasparini et al., 1999).

The discovery of subtype-specific group III mGlu receptor ligands opened new perspectives for studying the role of individual receptor subtypes and their importance as potential therapeutics (Lavreysen and Dautzenberg, 2008). Recently, the first selective and systemically active allosteric agonist of mGlu7 receptor, *N,N'-Bis*(diphenylmethyl)-1,2-ethanediamine dihydrochloride AMN082, has been identified (Mitsukawa et al., 2005), which up to 10 μM, it does not show inhibitory or activating effects at any of the other subtypes of mGlu receptors (Mitsukawa et al., 2005). Moreover, recently Suzuki et al. (2007) proposed 6-(4-Methoxyphenyl)-5-methyl-3-(4-pyridinyl)-isoxazolo[4,5-*c*]pyridin-4(5*H*)-one hydrochloride (MMPIP) as the first selective mGlu7 receptor antagonist, which at concentrations of at least 1 μM had no significant effect on mGluRs mGlu1, mGlu2, mGlu3, mGlu4, mGlu5, and mGlu8 receptors. Therefore, both AMN082 and MMPIP could be potentially useful as pharmacological tools for elucidating the role of mGlu7 receptors in neuroprotection in the central nervous system.

Anatomic evidence demonstrates that mGlu7 receptors has the highest CNS density of all group III mGluRs subtypes (Bradley et al., 1998; Shigemoto et al., 1997). The mGlu7 receptors are abundantly expressed in the neocortical regions, the cingulate and piriform cortex, hippocampus including CA1-CA3 and dentate gyrus, amygdala, locus coeruleus, and hypothalamic and thalamic nuclei (Bradley et al., 1996; Ohishi et al., 1995). Immunohistochemical and electron microscopy studies demonstrated that mGlu7 receptors are located predominantly near or within the active zone of presynaptic terminals on glutamate neurons, which suggests that mGlu7 receptors are autoreceptors serving a specific role in regulating glutamate release at the synapse (Shigemoto et al., 1996). Besides presynaptic, a postsynaptic distribution of mGluR7 has also been described in various brain regions (Bradley et al., 1996; Brandstätter et al., 1996; Kinzie et al., 1997; Kosinski et al., 1999). Moreover, it was found that activation of postsynaptic mGlu7 receptors reduced NMDAR-mediated currents and NMDAR surface expression via an actin-dependent mechanism (Gu et al., 2012, 2014).

Up until now, there have been very few studies on the neuroprotective effects of AMN082 in cellular and animal models (Jantas et al., 2014; Wang et al., 2012). Moreover, little is known about the role of group III mGlu receptor activation in neuroprotection against ischemic brain damage. Therefore, in the present study, we evaluated neuroprotective effects of the allosteric agonist of mGlu7 receptor, AMN082 in primary neuronal cultures in two in vitro models. In the first one, cortical cultures were exposed to oxygen-glucose deprivation (OGD), as a simple experimental model to study some aspects of ischemic-induced neurodegeneration (Goldberg and Choi, 1993), while in the second model, neuronal cell death was induced by KA in cortical and hippocampal neurons. Primary neuronal cell culture expresses mGlu7 receptors as has been shown previously for cortical neurons (Faden et al., 1997; Koga et al., 2010) as well as in our cortical and hippocampal neuronal cultures (Jantas et al., unpublished). The KA model was chosen as a good and validated simulation of various pathological effects of toxic glutamatergic overactivation that occurs, e.g. in ischemia (Coyle, 1983; Ferkany and Coyle, 1983; Wang et al., 2005), because KA acts not only directly via postsynaptic KA receptor stimulation, but also by the secondary massive release of endogenous glutamate which activates all the glutamatergic receptors and leads to neurodegeneration (Ferkany and Coyle, 1983; Ferkany et al., 1982). Moreover, the KA-induced neurodegeneration develops slowly, which makes it useful for studies of delayed neuroprotection (Mazzone and Nistri, 2011; Mazzone et al., 2010). Since preclinical studies have used very short time windows for drug administration, whereas a longer time window is permitted in clinical trials, in the present study we applied AMN082 at different time points, also after the insult, which makes our experiments more similar to the situation of the clinical practice.

2. Material and methods

2.1. Chemicals

N,N'-Bis(diphenylmethyl)-1,2-ethanediamine dihydrochloride (AMN082), 6-(4-Methoxyphenyl)-5-methyl-3-(4-pyridinyl)-isoxazolo[4,5-*c*]pyridin-4(5*H*)-one hydrochloride (MMPIP), MK-801 and MDL28170 were from Tocris Bioscience (Bristol, UK). Neurobasal A medium and supplement B27 were from Gibco (Invitrogen, Poisley, UK). The Cytotoxicity Detection Kit and BM Chemiluminescence Western Blotting Kit were from Roche Diagnostic (Mannheim, Germany). Primary antibodies: anti-spectrin α II (sc-48382), anti-β-actin (sc-47778), protein markers and appropriate secondary antibody were from Santa Cruz Biotechnology Inc. (CA,